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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
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L1             8 S "ZSIG63"
L2             5 DUP REM L1 (3 DUPLICATES REMOVED)
L3        230476 S SALIVA?
L4           622 S SECRETED (A)L3
L5           171 S HUMAN AND L4
L6       6615667 S CLON? OR EXPRESS? OR RECOMBINANT
L7            28 S L5 AND L6
L8            14 DUP REM L7 (14 DUPLICATES REMOVED)
L9        256214 S "ANTI-MICROBIAL" OR "ANTI-INFLAMMATORY" OR "IMMUNOMODULATORY"
L10            4 S L5 AND L9
L11            2 DUP REM L10 (2 DUPLICATES REMOVED)
                 E ADLER D A/AU
L12          240 S E3
                 E SHEPPARD P O/AU
L13          204 S E3
L14          439 S L12 OR L13
L15            2 S L5 AND L14
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=> s "zsig63"
L1 8 "ZSIG63"

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2 5 DUP REM L1 (3 DUPLICATES REMOVED)

=> d 1-5 ibib ab

L2 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
DUPLICATE 1
ACCESSION NUMBER: 2004-09527 BIOTECHDS
TITLE: Nucleic acid transfection composition useful for gene therapy and nucleic acid vaccine applications comprises a polyionic organic acid and nucleic acid;
involving recombinant vector-mediated gene transfer and expression in host cell gene therapy, recombinant vaccine and nucleic acid vaccine preparation
AUTHOR: BENNETT M J; CHEN Y; NIEDZINSKI E J; TSENG H; TUCKER S
PATENT ASSIGNEE: GENTERIC INC
PATENT INFO: WO 2004015089 19 Feb 2004
APPLICATION INFO: WO 2003-US25419 12 Aug 2003
PRIORITY INFO: US 2003-476145 4 Jun 2003; US 2002-402811 12 Aug 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-180663 [17]
AB DERWENT ABSTRACT:
NOVELTY - A nucleic acid transfection composition (I), comprising a polyionic organic acid and a nucleic acid, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) stabilizing (M1) a nucleic acid, by contacting the nucleic acid with a composition comprising a polyionic organic acid; (2) increasing (M2) the shelf life of a nucleic acid, by contacting the nucleic acid with a composition comprising a polyionic organic acid; and (3) neutralizing (M3) a virus, by administering a polyionic organic acid to an animal infected with the virus.
BIOTECHNOLOGY - Preferred Composition: In (I), the polyionic organic acid is a polyprotic polyaromatic organic compound. The polyionic organic acid is or is not a dye, or is suramin. The dye adsorbs radiation of the electromagnetic spectrum in the region chosen from ultraviolet region, visible region, infrared region and their combinations. The dye is a member chosen from acid dye, direct dye, reactive dye and disperse dye, preferably chosen from direct red, direct blue, direct yellow and direct

green. The dye is a member chosen from Light Blue, Congo Red and Evans Blue. The nucleic acid is chosen from DNA, RNA, DNA/RNA hybrid, antisense oligonucleotide, chimeric DNA-RNA polymer, ribozyme, viral vector DNA, and plasmid DNA. The nucleic acid encodes a peptide or protein which is immunogenic peptide or protein. The immunogenic peptide or protein is an antigen chosen from cancer antigen, bacterial antigen, viral antigen, fungal antigen, parasitic antigen, and antigen overexpressed on neoplastic cells. The peptide or protein is chosen from gamma glutamyl transpeptidase, manganese superoxide dismutase, metallothionein, glutathione peroxidase (GPx)-4, and catalase. The peptide or protein is chosen from IFN-alpha, IL-10, sTNFR, TGF-beta, IL-4, VIP, anti-TNF antibody, IL1-RA, other antibodies to proinflammatory cytokines, soluble gp39, soluble CD40, aquaporin-1, aquaporin-5, and **zsig63**. (I) is an aqueous solution. (I) further comprises an ionizable or ionized transition metal enhancer. (I) comprises aurine tricarboxylic acid (ATA) and ZnCl2 or Suramin and ZnCl2. The ionizable or ionized transition metal enhancer is a complex, adduct, cluster or salt of an element chosen from d-block element, first row f-block element, aluminum, and gallium, and is preferably zinc, nickel, cobalt, copper, aluminum and gallium. The ionizable or ionized transition metal enhancer is chosen from zinc sulfate, zinc acetate, nickel sulfate, nickel acetate, cobalt sulfate, cobalt acetate, copper sulfate, and copper acetate, preferably is zinc acetate or zinc sulfate. The ionizable or ionized transition metal enhancer is chosen from zinc halide, nickel halide, cobalt halide, copper halide, aluminum halide, and gallium halide, preferably chosen from ZnCl2, NiCl2, CoCl2, CuCl2, AlCl3, and GaCl3. (I) further comprises a secretory gland cell. (I) further comprises a member chosen from a cationic lipid, cationic polymer, and cationic peptide. (I) further comprises a nuclease inhibitor which is a DNase inhibitor chosen from DNase I inhibitor, DNase II inhibitor, divalent cation chelator, polymer, DNA binding agent, DNA nicking inhibitor, detergent, chaotropic salt, and an amino acid, preferably DNase I inhibitor or DNase II inhibitor. Preferred Method: In (M1), the nucleic acid is in a cell which is a secretory gland cell. The nucleic acid is DNA or plasmid DNA. The stabilization occurs in vivo or in vitro. The nucleic acid transfection composition increases the half-life of the nucleic acid. The composition increases the transfection efficiency for the nucleic acid. The composition increases the half life of the nucleic acid in a cell. In (M2), the increase in shelf life of a nucleic acid occurs in vitro. (M3) further involves administering a nucleic acid in combination with the polyionic organic acid or administering an ionizable or ionized transition metal enhancer in combination with the polyionic organic acid. (M3) further involves administering a nucleic acid. The ionizable or ionized transition metal enhancer is zinc chloride. The polyionic organic acid is a dye chosen from Congo Red, suramin, and aurintricarboxylic acid, preferably Congo Red. The virus is chosen from HIV, Epstein Bar virus, herpes simplex virus, hepatitis A, hepatitis B, hepatitis C, hepatitis E, mumps, measles, polio, and chicken pox, preferably HIV. The nucleic acid encodes a viral envelope protein. The polyionic organic acid neutralizes the virus by increasing the affinity of an antibody for the virus, by increasing the expression of the protein encoded by the nucleic acid or by directly inhibiting the virus.

ACTIVITY - Immunosuppressive; Antiarthritic; Antidiabetic; Dermatological; Antiinflammatory; Antithyroid; Antianemic; Antisickling; Hemostatic; Cardiovascular-Gen.; Hypotensive; Antilipemic; Antianginal; Antiparkinsonian; Nootropic; Neuroprotective; Gastrointestinal-Gen.; CNS-Gen.; Respiratory-Gen.; Anorectic; Antibacterial; Virucide; Hepatotropic; Fungicide; Antiparasitic; Cytostatic; Antiasthmatic; Anti-HIV.

MECHANISM OF ACTION - Gene therapy; Nucleic acid vaccine; Direct inhibitor of viruses. 88 microg DNA encoding HIV envelope protein gp120 in 200 microl distilled, deionized water was retroductally delivered (50 microl/minute) using a standard syringe pump to the submandibular salivary glands of Sprague Dawley rats on weeks 0 and 3. The DNA was

delivered in a formulation comprising (DOHBD):distearoylphosphatidylethanolamine (DOPE) lipid (3:1)/Zinc (0.125 mM), Congo Red (6 mg/ml), Evans Blue (6 mg/ml), or Congo Red (6 mg/ml)/DOHBD:DOPE lipid (3:1)/Zn (0.125 mM). Anti-gp120 IgG titer was measured by enzyme linked immunosorbant assay (ELISA) over 17 weeks. All formulations were able to generate significant antibody responses to gp120 protein, with the Zn/lipid formulation generating the lowest IgG titer compared to the other formulations. On week 9, plasma samples were collected and HIV neutralization assays were performed using HIV strains Bal and MN. Results showed neutralization of Bal and MN.

USE - (I) is useful for administering a nucleic acid to a cell which involves contacting the cell with (I). The cell is a secretary gland cell which is chosen from salivary gland cell, pancreatic cell, mammary gland cell, thyroid cell, thymus cell, pituitary gland cell, and a liver cell. The secretory gland cell is a salivary gland cell. The peptide or protein encoded by the nucleic acid is secreted or released from the secretory gland cell. (I) is delivered to the cell by electroporation administration, ultrasound administration and ionophoresis administration. (I) acts by perturbing the cell membrane. The transfection occurs in vivo or in vitro. (I) is useful for increasing transfection efficiency in a cell which involves contacting a cell with (I). The cell is a secretory gland cell. The peptide or protein remains in the secretory gland cell. The peptide or protein is secreted locally or systemically from the secretory gland cell. The nucleic acid is localized in the cell. The nucleic acid is operably linked to an expression control sequence which is tissue specific. The tissue is intestinal epitheliums. The tissue is liver. The nucleic acid encodes a therapeutic protein. The therapeutic protein is not or is expressed in an intestinal cell. The therapeutic protein is expressed in an intestinal epithelial cell (all claimed). (I) is useful for stabilizing a nucleic acid. (I) is useful for preventing or treating autoimmune disorders such as arthritis, diabetes, systemic lupus erythematosus, or Grave's disease, blood disorders such as anemia, sickle cell anemia, globin disorder, or clotting disorder such as hemophilia, cardiovascular disorders such as high blood pressure, high cholesterol, angina, central nervous system disorders such as Parkinson's disease, Alzheimer's disease, multiple sclerosis and Lou Gehrig's disease, gastrointestinal disorders such as esophageal reflux, lactose deficiency, metabolic disorders such as obesity, lysosomal storage disease, Hurler's disease, Hunter's disease, neoplastic diseases such as colon cancer, stomach cancer, liver cancer, etc., pulmonary disorders such as cystic fibrosis, emphysema or asthma, bacterial diseases such as diphtheria, Lyme disease, meningitis, etc., fungal and parasitic diseases, and viral diseases such as those caused by HIV, Epstein Barr virus, herpes simplex virus, hepatitis A, etc.

ADMINISTRATION - (I) is delivered to the cell by retroductal delivery or direct administration. The administering is by cannulation or injection (claimed). No dosage details are given.

ADVANTAGE - The nucleic acid of (I) has an increased half-life compared to nucleic acid alone (claimed). Polyionic organic acids of (I) enhances in vivo salivary gland transfection efficiency. (I) stabilizes nucleic acid. (91 pages)

L2 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-13605 BIOTECHDS
TITLE: Novel isolated **zsig63** polypeptide, member of the adhesin family, useful for treating dental carries, periodontal disease, thrush, gastrointestinal disease, urinary tract infections, vaginal infections, skin infections ;

vector-mediated gene transfer and expression in host cell for recombinant protein production for use in disease diagnosis and gene therapy

AUTHOR: ADLER D A; SHEPPARD P O
PATENT ASSIGNEE: ADLER D A; SHEPPARD P O

PATENT INFO: US 2002173027 21 Nov 2002
APPLICATION INFO: US 2001-922469 3 Aug 2001
PRIORITY INFO: US 2001-922469 3 Aug 2001; US 1999-124820 17 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-328428 [31]

AB DERWENT ABSTRACT:

NOVELTY - An isolated **zsig63** polypeptide (I) comprising at least 90 % identity to an amino acid sequence which comprises amino acids 16(Arg)-37(Ser) (domain 1 of **zsig63**), 38(Leu)-126(Ala) (domain 2), 127(Pro)-219(Gln) (domain 3), 16(Arg)-219(Gln) (mature **zsig63** polypeptide) or 1(Met)-219(Gln) of a 219 amino acid sequence (S1), given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated polynucleotide (II) encoding (I); (2) an expression vector (III) comprising the following operably linked elements, a transcription promoter, DNA segment encoding a **zsig63** polypeptide comprising an amino acid sequence that is at least 90 % identical to amino acids 16(Arg)-219(Gln) of (S1), and a transcription terminator; (3) a cultured cell (IV) into which has been introduced (III), where the cell expresses a polypeptide encoded by the DNA segment; (4) a DNA construct (V) encoding a fusion protein, comprising a first DNA segment encoding a polypeptide chosen from amino acid sequence (S1) from residues 1(Met)-15(Ala), 16(Arg)-37(Ser), 38(Leu)-126(Ala), 127(Pro)-219(Gln), 16(Arg)-219(Gln), and at least one other DNA segment encoding an additional polypeptide, where the first and other DNA segments are connected in-frame, and encode the fusion protein; (5) a fusion protein (VI) produced by culturing a host cell into which has been introduced a vector comprising operably linked transcriptional promoter, (V), and transcriptional terminator, and recovering the protein encoded by the DNA segment; (6) producing (I); (7) detecting in a test sample, the presence of agonist of **zsig63** protein activity, comprising: (a) transfecting a **zsig63**-responsive cell with a reporter gene construct that is responsive to **zsig63**-stimulated cellular pathway, adding a test sample; and (b) comparing levels of response in the presence and absence of the test sample, by a biological or biochemical assay, and determining from the comparison, the presence of the agonist of **zsig63** activity in the sample; (8) producing an antibody to **zsig63** polypeptide, which involves inoculating an animal with a polypeptide consisting of 9-204 amino acids, which consists of a contiguous sequence of amino acids in (S1) from amino acids 16(Ala)-219(Gln), (I), and a polypeptide comprising amino acids 16(Arg)-37(Ser), 38(Leu)-126(Ala), 127(Pro)-219(Gln), 16(Arg)-219(Gln), 1(Met)-219(Gln), 14(Phe)-19(Arg), 16(Arg)-21(Phe), 24(Gly)-29(Asp), 25(Glu)-30(Asp), 187(Glu)-192(Glu), 24(Gly)-33(Pro), 17(Lys)-33(Pro), 66(Thr)-73(Pro), 103(Pro)-108(Gly), 190(Ala)-197((Glu), 202(Lys)-215(Gly) or 190(Ala)-215(Glu) of (S1), where the polypeptide elicits an immune response in the animal to produce the antibody, and isolating the antibody from the animal; (9) an antibody (VII) produced by the method of (8), which binds to (I); and (10) an antibody (VIII) that binds to (I).

WIDER DISCLOSURE - (1) counterpart polypeptides and polynucleotides of **zsig63**; (2) allelic and splice variants of (S1) and (S2); (3) functional fragments of (I) and the polynucleotides encoding the fragments; (4) identifying agonist and antagonist of **zsig63** polypeptide using a microphysiometer; and (5) mice engineered to express **zsig63** gene referred to as transgenic mice, and mice that exhibit complete absence of **zsig63** gene function referred to as knockout mice.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) and isolating the polypeptide produced by the cell (claimed). Preferred Polynucleotide: (I), preferably comprises an amino acid sequence which comprises amino acid residues 16(Arg)-37(Ser), 38(Leu)-126(Ala), 127(Pro)-219(Gln), 16(Arg)-219(Gln) or 1(Met)-219(Gln) of (S1). (II) nucleotides 173-784 or 128-784 of a 1008 nucleotide sequence (S2), given

in the specification, or a polynucleotide sequence complementary to the sequence. Optionally, (II) comprises nucleotides 1-657 of a fully defined degenerate sequence of (S1) which has 657 nucleotides, given in the specification. Preferred Vector: (III) further comprises a secretory signal sequence operably linked to the DNA segment. Preferred Antibody: (VII) is a monoclonal antibody.

ACTIVITY - Antimicrobial; Antibacterial; Vulnerary. No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - (I) is useful for detecting in a test sample, the presence of antagonist of **zsig63** protein activity, which involves transfecting a **zsig63**-responsive cell with a reporter gene construct that is responsive to **zsig63**-stimulated cellular pathway, and producing (I) by recombinant techniques, and adding (I) to the cell in the presence and absence of the test sample, and comparing level of response to the polypeptide in the presence and absence of the test sample, by a biological or biochemical assay, and determining from the comparison, the presence of the antagonist of **zsig63** activity in the sample. (IV) is useful for producing (I) by standard recombinant methods. (All claimed.) (I) comprises 16 full evenly-spaced coil-like repeats in domain 2. The coil-like repeats are useful for identifying new family members. (I) and (II) are useful for identifying and isolating receptors that bind to **zsig63** polypeptide. (I) has antimicrobial activity and since exhibits high expression in salivary gland, can be used for treating dental carries, periodontal disease, thrush, and gastrointestinal disease, urinary tract infections, vaginal infections, skin infections and other epithelial wounds. The polypeptides can be used to establish normal microflora and protect against pathogenic colonization and invasion. (I) can also be used for providing pro-inflammatory activity for treating chronic, tissue damage particularly in areas having limited or damaged vascular system, e.g. damage in extremities associated with diabetes. (I) is also useful for treating conditions where stimulation of immune responsiveness is desired, e.g. AIDS patient or in individuals that have undergone chemotherapy, radiation treatment, etc. (I) is also useful for treating lung infections associated with cystic fibrosis. (I) is useful for studying chemoattraction of monocytes in cell culture, studying activity of melanocortin family of receptors in cell culture, studying ion flux in cell culture, studying cytocidal activity in mammalian cell such as tumor cells in cell culture, as cell culture reagents in in vitro studies of exogenous microorganism infection such as bacterial, viral or fungal infections, and for studying epithelial cell defensin induction in cell culture. (I) is useful as a diagnostic reagent e.g. **zsig63** polypeptide is detected in the serum or tissue biopsy of a patient, for evaluating salivary gland function or dysfunction (e.g. digestive dysfunction, wound healing dysfunction, inadequate saliva production or composition, mucosal integrity breakdown, and failure or diminished anti-microbial function. Detection of **zsig63** polypeptide at relatively high levels in the trachea may indicate that such polypeptides may serve as a marker of lung dysfunction. (I) is also useful as a diagnostic reagent for conditions associated with salivary gland or lung dysfunction including salivary gland carcinoma, Pneumocystis carinii infection (particularly associated with AIDS patient), emphysema, chronic bronchitis, etc. (I) is also useful for diagnosing prostate dysfunctions such as prostate adenocarcinoma. (I) is useful for aiding digestion, and as components of defined cell culture media and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in culture. The **zsig63** polypeptides are useful as research reagent such as for the expansion of cultured cells, and as immunogen to prepare anti-**zsig63** antibodies. (II) is useful in gene therapy applications to increase or inhibit **zsig63** activity, and for detecting abnormalities on human chromosome 4 associated with disease or other human traits. **Zsig63** polynucleotide probes can be used to detect abnormalities or genotypes

associated with genes located at the 4q12-4q13 region of chromosome 4, e.g. dentinogenesis imperfecta, and dentin dysplasia type II.

ADMINISTRATION - (I) is administered by topical, inhalant, parenteral, preferably intravenous or subcutaneous delivery. No dosages is given.

EXAMPLE - Scanning of a translated DNA database resulted in identification of an expressed sequence tag (EST) sequence found to be a novel member of the adhesin family and designated **zsig63**. Confirmation of the EST sequence was made by sequence analyses of the cDNA from which the EST originated. This cDNA clone was obtained and sequenced. Northern blot tissue distribution of the mRNA revealed high expression in salivary gland, and moderate to high expression in thyroid. (32 pages)

L2 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-01837 BIOTECHDS
TITLE: Novel secreted salivary polypeptide, **zsig63**, useful as antimicrobial agent for treating microbial infection, dental carries, periodontal disease, thrush gastrointestinal disease, and for aiding digestion; recombinant protein production and agonist and antagonist use in disease therapy and gene therapy
AUTHOR: ADLER D A; SHEPPARD P O
PATENT ASSIGNEE: ADLER D A; SHEPPARD P O
PATENT INFO: US 2002090677 11 Jul 2002
APPLICATION INFO: US 2001-923236 3 Aug 2001
PRIORITY INFO: US 2001-923236 3 Aug 2001; US 1999-124820 17 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-642378 [69]
AB DERWENT ABSTRACT:

NOVELTY - An isolated secreted salivary polypeptide (I) designated as **zsig63**, comprising an amino acid sequence 90% identical to a sequence (S1) of 1 (Met)-219 (Gln) fully defined in the specification, 16 (Arg)-37 (Ser), 38 (Leu)-126 (Ala), 127 (Pro)-219 (Gln) or 16 (Arg)-219 (Gln) of (S1), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I); (2) an expression vector (III) comprising a transcription promoter, a DNA segment encoding a **zsig63** polypeptide comprising an amino acid sequence that is 90% identical to (S1) from amino acid 16 (Arg)-219 (Gln) and a transcription terminator, operably linked to each other; (3) a cultured cell (IV) containing (III), expresses a polypeptide encoded by the DNA segment; (4) a DNA construct (V) encoding a fusion protein, comprises a first DNA segment encoding a polypeptide comprising an amino acid sequence of 1 (Met)-15 (Ala), 16 (Arg)-37 (Ser), 38 (Leu)-126 (Ala), 127 (Pro)-219 (Gln) or 16 (Arg)-219 (Gln) of (S1), and at least one other DNA segment encoding an additional polypeptide, where the first and other DNA segments are connected in-frame and encode the fusion protein; (5) a fusion protein produced by culturing a host cell comprising a vector having transcriptional promoter, (V), a transcriptional terminator, operably linked to each other and recovering the protein encoded by the DNA segment, is introduced; (6) producing (I); (7) detecting in a test sample, the presence of an agonist or antagonist of **zsig63** protein activity, involves transfecting a **zsig63**-responsive cell, with a reporter gene construct that is responsive to a **zsig63**-stimulated cellular pathway and producing a **zsig63** polypeptide by the culturing the cell, and adding the zig63 polypeptide to the cell in the presence and absence of a test sample and comparing levels of response to the **zsig63** polypeptide, in the presence and absence of the test sample, by a biological or biochemical assay and determining from the comparison, the presence of the agonist or antagonist of **zsig63** activity in the test sample; (8) producing an antibody to **zsig63** polypeptide, involves inoculating animal

with polypeptides consisting of 9 to 204 amino acids, the polypeptide consist of a contiguous sequence of amino acids in (S1) from amino acid 16 (Ala) to amino acid number 219 (Gln), (I), or a polynucleotide comprising 14 (Phe)-19 (Arg), 16 (Arg)-21 (Phe), 24 (Gly)-29(Asp), 25 (Glu)-30(Asp), 187 (Glu)-192 (Glu), 24 (Gly)-33 (Pro), 17 (Lys)-33 (Pro), 66 (Thr)-73 (Pro), 102 (Pro)-108 (Gly), 180 (Ala)-197 (Glu), 202 (Lys)-215 (Gly), 190 (Ala)-215 (Glu) of (S1), where the polypeptide elicits an immune response in the animal to produce the antibody, and isolating the antibody from the animal; and (9) an antibody (Ab) produced by the above method which binds to a **zsig63** polypeptide or (I).

WIDER DISCLOSURE - Also disclosed are: (1) a zig63 polypeptide-encoding polynucleotides comprising a sequence of 657 nucleotides fully defined in the specification; (2) fragments of a sequence of 1008 nucleotides fully defined in the specification; (3) orthologs, variant or functional fragment of (I) and (II); (4) nucleic acid molecule encoding functional fragment of (I); (5) a pharmaceutical composition comprising (I); and (6) reagent comprising **zsig63** gene, a probe comprising **zsig63** DNA or RNA or a subsequence for diagnostic application.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) and isolating (I) produced by the cell. Preferred Vector: (III) further comprises a secretory signal sequence operably linked to the DNA segment. Preferred Antibody: Ab is a monoclonal antibody.

ACTIVITY - Antibacterial; Fungicide; Virucide; Antiinflammatory; Antiarteriosclerotic; Vasotropic; Anorectic. No biological data provided.

MECHANISM OF ACTION - Gene therapy; Modulator of (I). No supporting data provided.

USE - (I) is useful as a host defense polypeptide, immune modulating factor, antipathogenic polypeptide, cell-cell signaling molecule, growth factor, cytokine, or as secreted extracellular matrix associated proteins with growth factor hormone activity. (I) is useful for treating conditions associated with pathological microbes, including bacterial, fungal, and viral infections, for treating dental carries (tooth decay), periodontal disease, thrush, and gastrointestinal disease, for treating urinary tract infection, vaginal infection, and for preventing infection in skin and other epithelial wounds. (I) is useful for establishing normal microflora and protect against pathogenic colonization and invasion, for treating chronic tissue damage e.g. damage in extremities associated with diabetes, and useful as anti-inflammatory agents. (I) is useful as a marker of lung dysfunction, salivary gland dysfunction, or dysfunction of prostate gland. (I) is therapeutically useful for aiding digestion. (I) is useful as a research reagent for the expansion of cultured cells, to prepare antibodies that bind to **zsig63** epitopes, peptides or polypeptides, as antigen to inoculate an animal for eliciting an immune response. **zsig63** is useful for identifying cells, tissues or cell lines which respond to **zsig63**-stimulated pathway, or for identifying inhibitors of its activity. (I) is useful for studies to isolate mesenchymal stem cells and myocyte or other progenitor cells both in vivo and ex vivo, and serve as an additional cell surface or secreted marker associated with stage-specific expression of a tissue. (II) is useful in gene therapy for increasing or inhibiting **zsig63** activity, for detecting abnormalities on human chromosome 4 associated with disease or other human traits and as diagnostics in forensic DNA profiling. (I) or Ab is useful for studying chemoattraction of monocytes, for studying the activity of the melanocortin family of receptors, for studying or evaluating ligand or putative ligand binding and/or ion flux (calcium flux, potassium flux, sodium flux) regulation or modulation, for studying cytocidal activity against mammalian cells, as cell culture reagent in in vitro studies of exogenous microorganism infection, and in in vivo animal models of infection. (I) or Ab is useful for identifying and isolating receptors for **zsig63**. Ab is useful for detecting **zsig63** polypeptides in the serum or tissue biopsy of a patient undergoing evaluation for salivary gland function or dysfunction. Ab is useful for tagging cells that express **zsig63**

, for isolating **zsig63** for diagnostic assays for determining circulating levels of **zsig63** polypeptides, for detecting or quantitating soluble **zsig63** as marker of underlying pathology or disease, in analytical methods employing fluorescence activated cell sorting (FACS), for screening expression libraries, for generating anti-idiotypic antibodies, as neutralizing antibodies or as antagonists to block **zsig63** activity in vitro or in vivo, and in in vitro detection of denatured **zsig63** or its fragments in assays. (I), (II) or Ab is useful for stimulating proliferation or differentiation of cardiac myocytes, for proliferation or differentiation of adipocytes and for inhibiting chondrosarcomas, atherosclerosis, restenosis and obesity.

ADMINISTRATION - Pharmaceutical composition comprising (I) is administered through topical, inhalation, parenteral, intravenous or subcutaneous route. Dosage not specified.

EXAMPLE - Full-length **zsig63** was obtained using an expressed sequence tag (EST) sequence. Scanning of a translated DNA database resulted in identification of EST sequence found to be a novel member of the adhesion family and designated **zsig63**. Confirmation of EST sequence was made by sequence analyses of the cDNA from the EST originated. This cDNA clone was obtained and sequenced using the ZC6768 (gcaattaaccctcactaaagggaac), ZC694 (taatacgactcactataggg), ZC7231 (ttttttttttttttttttttttttttv), ZC7764a (ttttttttttttttttttttttttta). The insert was about 1 kb and was full-length. (33 pages)

L2 ANSWER 4 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-01808 BIOTECHDS
TITLE: Novel secreted salivary protein, **zsig63** and polynucleotide encoding it useful for treating microbial infections, inflammatory conditions, dental caries and lung infections associated with cystic fibrosis; vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy
AUTHOR: ADLER D A; SHEPPARD P O
PATENT ASSIGNEE: ADLER D A; SHEPPARD P O
PATENT INFO: US 2002081701 27 Jun 2002
APPLICATION INFO: US 2001-922480 3 Aug 2001
PRIORITY INFO: US 2001-922480 3 Aug 2001; US 1999-124820 17 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-635468 [68]
AB DERWENT ABSTRACT:

NOVELTY - An isolated **zsig63** (I), a secreted salivary protein comprising a sequence of amino acid residues that is at least 90% identical to a sequence (S1) of 219 amino acids given in the specification, or amino acids 16-37, 38-126, 127-219 or 16-219 of (S1), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I); (2) an expression vector (III) comprising a DNA segment encoding **zsig63** polypeptide comprising an amino acid sequence that is 90% identical to amino acids 16-219 of (S1), and transcription promoter and terminator; (3) a cultured cell (IV) into which has been introduced (III), where the cell expresses a polypeptide encoded by the DNA segment; (4) a DNA construct encoding a fusion protein, comprising a first DNA segment encoding amino acids 1-15, 16-37, 38-126, 127-219 or 16-219 of (S1), and at least one other DNA segment encoding an additional polypeptide, where the first and other DNA segments are connected in-frame and encode the fusion protein; (5) a fusion protein produced by culturing a host cell into which has been introduced a vector comprising the above DNA construct and transcription promoter and terminator; (6) producing **zsig63** polypeptide; and (7) an antibody (V) produced using (I), that binds to (I).

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) and isolating the polypeptide produced by the cell (claimed). Preferred Polypeptide: (I) comprises amino acids 16-37, 38-126, 127-219, 16-219 or 1-219 of (S1). Preferred Polynucleotide: (II) comprises a polynucleotide sequence (or its complement) comprising nucleotides 173-784 or 128-784 of a sequence (S2) of 1008 bp given in the specification. Preferred Vector: (III) further comprises a secretory signal sequence operably linked to the DNA segment. Preferred Antibody: (V) is a monoclonal antibody.

ACTIVITY - Antibacterial; Fungicide; Virucide; Vulnerary; Anti-HIV; Antiinflammatory. No supporting data given.

MECHANISM OF ACTION - Gene therapy. No supporting data given.

USE - (I) is useful for detecting in a test sample, the presence of an antagonist or agonist of **zsig63** protein activity. The method comprises transfecting a **zsig63**-responsive cell with a reporter gene construct that is responsive to a **zsig63**-stimulated cellular pathway, adding the **zsig63** polypeptide to the cell, in the presence and absence of the test sample, comparing the levels of response to the **zsig63** polypeptide in the presence and absence of the test sample, by a biological or biochemical assay, and determining from the comparison, the presence of the antagonist or agonist of **zsig63** activity in the test sample. (I) is also useful as an immunogen for producing an antibody to **zsig63** polypeptide, by inoculating an animal with (I), or its fragment comprising amino acids 9-204, 14-19, 16-21, 24-29, 25-30, 187-192, 24-33, 17-33, 66-73, 103-108, 190-197, 202-215, or 190-215 of (S1) and isolating the antibody from the animal (all claimed). **zsig63**-cytokine fusion proteins or antibody-cytokine fusion protein are useful for enhancing in vivo killing of target tissues. Pharmaceutical composition comprising purified **zsig63** polypeptide are useful in the treatment of conditions associated with pathological microbes, including bacterial, fungal and viral infections. High expression of **zsig63** in salivary gland suggests that anti-microbial polypeptides are useful for treatment of dental caries (tooth decay), periodontal disease, thrush and gastrointestinal disease. Other applications can be used in urinary tract infections, vaginal infections, prevention of infection in skin and other epithelial wounds. The polypeptides can be used to establish normal microflora and protect against pathogenic colonization and invasion. (I) is useful when pro-inflammatory activity is desired. Applications for such pro-inflammatory activity include the treatment of chronic tissue damage, particularly in areas having a limited or damaged vascular system e.g., damage in extremities associated with diabetes. Antagonists to **zsig63** polypeptides may be useful as anti-inflammatory agents. (I) is useful for the treatment of patients having incompetent immune system, such as AIDS patients or individuals that have undergone chemotherapy, radiation treatment. (I) is also useful for the treatment of lung infections associated with cystic fibrosis, for studying chemoattraction of monocytes in cell culture, for studying activity of the melanocortin family of receptors and ion flux in cell culture, and cytocidal activity against mammalian cells. (I), its fragments, fusion proteins, or agonists are useful as cell culture reagents in in vitro studies of exogenous microorganism infection, such as bacterial, viral or fungal infection and also in in vivo animal models of infection. (I), its agonists or antagonists are useful for aiding digestion. **zsig63** is also useful to identify cells, tissues or cell lines which respond to a **zsig63**-stimulated pathway, to identify and isolate receptors for **zsig63** and in diagnostic applications. The diagnostic methods are useful in genetic linkage analysis, to detect a genetic abnormality or aberration in a patient. (V) is useful for detecting **zsig63** polypeptides.

ADMINISTRATION - Administered by topical, inhalant or parenteral, particularly intravenous or subcutaneous route. Dosage not specified.

EXAMPLE - Scanning of a translated DNA database resulted in identification of an expressed sequence tag (EST) sequence found to be a novel member of the adhesion family and designated **zsig63**.

Confirmation of the EST sequence was made by sequence analyses of 25 the cDNA from which the EST originated. This cDNA clone was obtained and sequenced using the following primers ZC6768 (gcaattaaccctcactaaagggaac, ZC694 (taatacgactcactataggg), ZC7231(ttttttttttttttttttttttttttv) and ZC7764a (ttttttttttttttttttttttttta). The insert was about 1 kb and was full-length. **Zsig63** was mapped to chromosome 4. (33 pages)

L2 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
ACCESSION NUMBER: 2002:143438 BIOSIS
DOCUMENT NUMBER: PREV200200143438
TITLE: Secreted salivary **ZSIG63** Polypeptide.
AUTHOR(S): Adler, David A. [Inventor, Reprint author]; Sheppard, Paul O. [Inventor]
CORPORATE SOURCE: Bainbridge Island, WA, USA
ASSIGNEE: ZymoGenetics, Inc.
PATENT INFORMATION: US 6331413 December 18, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 18, 2001) Vol. 1253, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Feb 2002
Last Updated on STN: 26 Feb 2002
AB The present invention relates to polynucleotide and polypeptide molecules for **zsig63**, a novel secreted salivary protein. The polypeptides, and polynucleotides encoding them, may exhibit anti-microbial activity and may be used in the study or treatment of microbial infections. The polynucleotides encoding **zsig63**, are located on chromosome 4, and can be used to identify a region of the genome associated with human disease states. The present invention also includes antibodies to the **zsig63** polypeptides.

```
=> s saliva?
L3      230476 SALIVA?

=> s secreted (a)l3
L4         622 SECRETED (A) L3

=> d his

     (FILE 'HOME' ENTERED AT 15:56:47 ON 20 JUL 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 15:57:13 ON 20 JUL 2004
L1            8 S "ZSIG63"
L2            5 DUP REM L1 (3 DUPLICATES REMOVED)
L3       230476 S SALIVA?
L4          622 S SECRETED (A)L3

=> s human and l4
L5         171 HUMAN AND L4

=> s clon? or express? or recombinant
   5 FILES SEARCHED...
L6     6615667 CLON? OR EXPRESS? OR RECOMBINANT

=> s l5 and l6
L7          28 L5 AND L6

=> dup rem l7
PROCESSING COMPLETED FOR L7
```

L8 14 DUP REM L7 (14 DUPLICATES REMOVED)

=> d 1-14 ibib ab

L8 ANSWER 1 OF 14 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-01837 BIOTECHDS
TITLE: Novel **secreted salivary** polypeptide, zsig63, useful as antimicrobial agent for treating microbial infection, dental carries, periodontal disease, thrush gastrointestinal disease, and for aiding digestion; **recombinant** protein production and agonist and antagonist use in disease therapy and gene therapy
AUTHOR: ADLER D A; SHEPPARD P O
PATENT ASSIGNEE: ADLER D A; SHEPPARD P O
PATENT INFO: US 2002090677 11 Jul 2002
APPLICATION INFO: US 2001-923236 3 Aug 2001
PRIORITY INFO: US 2001-923236 3 Aug 2001; US 1999-124820 17 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-642378 [69]
AB DERWENT ABSTRACT:

NOVELTY - An isolated **secreted salivary** polypeptide (I) designated as zsig63, comprising an amino acid sequence 90% identical to a sequence (S1) of 1 (Met)-219 (Gln) fully defined in the specification, 16 (Arg)-37 (Ser), 38 (Leu)-126 (Ala), 127 (Pro)-219 (Gln) or 16 (Arg)-219 (Gln) of (S1), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I); (2) an **expression** vector (III) comprising a transcription promoter, a DNA segment encoding a zsig63 polypeptide comprising an amino acid sequence that is 90% identical to (S1) from amino acid 16 (Arg)-219 (Gln) and a transcription terminator, operably linked to each other; (3) a cultured cell (IV) containing (III), **expresses** a polypeptide encoded by the DNA segment; (4) a DNA construct (V) encoding a fusion protein, comprises a first DNA segment encoding a polypeptide comprising an amino acid sequence of 1 (Met)-15 (Ala), 16 (Arg)-37 (Ser), 38 (Leu)-126 (Ala), 127 (Pro)-219 (Gln) or 16 (Arg)-219 (Gln) of (S1), and at least one other DNA segment encoding an additional polypeptide, where the first and other DNA segments are connected in-frame and encode the fusion protein; (5) a fusion protein produced by culturing a host cell comprising a vector having transcriptional promoter, (V), a transcriptional terminator, operably linked to each other and recovering the protein encoded by the DNA segment, is introduced; (6) producing (I); (7) detecting in a test sample, the presence of an agonist or antagonist of zsig63 protein activity, involves transfecting a zsig63-responsive cell, with a reporter gene construct that is responsive to a zsig63-stimulated cellular pathway and producing a zsig63 polypeptide by the culturing the cell, and adding the zig63 polypeptide to the cell in the presence and absence of a test sample and comparing levels of response to the zsig63 polypeptide, in the presence and absence of the test sample, by a biological or biochemical assay and determining from the comparison, the presence of the agonist or antagonist of zsig63 activity in the test sample; (8) producing an antibody to zsig63 polypeptide, involves inoculating animal with polypeptides consisting of 9 to 204 amino acids, the polypeptide consist of a contiguous sequence of amino acids in (S1) from amino acid 16 (Ala) to amino acid number 219 (Gln), (I), or a polynucleotide comprising 14 (Phe)-19 (Arg), 16 (Arg)-21 (Phe), 24 (Gly)-29(Asp), 25 (Glu)-30(Asp), 187 (Glu)-192 (Glu), 24 (Gly)-33 (Pro), 17 (Lys)-33 (Pro), 66 (Thr)-73 (Pro), 102 (Pro)-108 (Gly), 180 (Ala)-197 (Glu), 202 (Lys)-215 (Gly), 190 (Ala)-215 (Glu) of (S1), where the polypeptide elicits an immune response in the animal to produce the antibody, and isolating the antibody from the animal; and (9) an antibody (Ab) produced by the above method which binds to a zsig63 polypeptide or (I).

WIDER DISCLOSURE - Also disclosed are: (1) a zig63 polypeptide-encoding polynucleotides comprising a sequence of 657 nucleotides fully defined in the specification; (2) fragments of a sequence of 1008 nucleotides fully defined in the specification; (3) orthologs, variant or functional fragment of (I) and (II); (4) nucleic acid molecule encoding functional fragment of (I); (5) a pharmaceutical composition comprising (I); and (6) reagent comprising zsig63 gene, a probe comprising zsig63 DNA or RNA or a subsequence for diagnostic application.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) and isolating (I) produced by the cell. Preferred Vector: (III) further comprises a secretory signal sequence operably linked to the DNA segment. Preferred Antibody: Ab is a monoclonal antibody.

ACTIVITY - Antibacterial; Fungicide; Virucide; Antiinflammatory; Antiarteriosclerotic; Vasotropic; Anorectic. No biological data provided.

MECHANISM OF ACTION - Gene therapy; Modulator of (I). No supporting data provided.

USE - (I) is useful as a host defense polypeptide, immune modulating factor, antipathogenic polypeptide, cell-cell signaling molecule, growth factor, cytokine, or as secreted extracellular matrix associated proteins with growth factor hormone activity. (I) is useful for treating conditions associated with pathological microbes, including bacterial, fungal, and viral infections, for treating dental carries (tooth decay), periodontal disease, thrush, and gastrointestinal disease, for treating urinary tract infection, vaginal infection, and for preventing infection in skin and other epithelial wounds. (I) is useful for establishing normal microflora and protect against pathogenic colonization and invasion, for treating chronic tissue damage e.g. damage in extremities associated with diabetes, and useful as anti-inflammatory agents. (I) is useful as a marker of lung dysfunction, salivary gland dysfunction, or dysfunction of prostate gland. (I) is therapeutically useful for aiding digestion. (I) is useful as a research reagent for the expansion of cultured cells, to prepare antibodies that bind to zsig63 epitopes, peptides or polypeptides, as antigen to inoculate an animal for eliciting an immune response. zsig63 is useful for identifying cells, tissues or cell lines which respond to zsig63-stimulated pathway, or for identifying inhibitors of its activity. (I) is useful for studies to isolate mesenchymal stem cells and myocyte or other progenitor cells both in vivo and ex vivo, and serve as an additional cell surface or secreted marker associated with stage-specific **expression** of a tissue. (II) is useful in gene therapy for increasing or inhibiting zsig63 activity, for detecting abnormalities on **human** chromosome 4 associated with disease or other **human** traits and as diagnostics in forensic DNA profiling. (I) or Ab is useful for studying chemoattraction of monocytes, for studying the activity of the melanocortin family of receptors, for studying or evaluating ligand or putative ligand binding and/or ion flux (calcium flux, potassium flux, sodium flux) regulation or modulation, for studying cytocidal activity against mammalian cells, as cell culture reagent in in vitro studies of exogenous microorganism infection, and in in vivo animal models of infection. (I) or Ab is useful for identifying and isolating receptors for zsig63. Ab is useful for detecting zsig63 polypeptides in the serum or tissue biopsy of a patient undergoing evaluation for salivary gland function or dysfunction. Ab is useful for tagging cells that **express** zsig63, for isolating zsig63 for diagnostic assays for determining circulating levels of zsig63 polypeptides, for detecting or quantitating soluble zsig63 as marker of underlying pathology or disease, in analytical methods employing fluorescence activated cell sorting (FACS), for screening **expression** libraries, for generating anti-idiotypic antibodies, as neutralizing antibodies or as antagonists to block zsig63 activity in vitro or in vivo, and in in vitro detection of denatured zsig63 or its fragments in assays. (I), (II) or Ab is useful for stimulating proliferation or differentiation of cardiac myocytes, for proliferation or differentiation of adipocytes and for inhibiting chondrosarcomas,

atherosclerosis, restenosis and obesity.

ADMINISTRATION - Pharmaceutical composition comprising (I) is administered through topical, inhalation, parenteral, intravenous or subcutaneous route. Dosage not specified.

EXAMPLE - Full-length zsig63 was obtained using an **expressed** sequence tag (EST) sequence. Scanning of a translated DNA database resulted in identification of EST sequence found to be a novel member of the adhesion family and designated zsig63. Confirmation of EST sequence was made by sequence analyses of the cDNA from the EST originated. This cDNA **clone** was obtained and sequenced using the ZC6768 (gcaattaaccctcactaaagggaac), ZC694 (taatacgactcactataggg), ZC7231 (ttttttttttttttttttttttttv), ZC7764a (ttttttttttttttttttttttttta). The insert was about 1 kb and was full-length. (33 pages)

L8 ANSWER 2 OF 14 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2002261135 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12000641
TITLE: **Expression** of D7 and D7-related proteins in the salivary glands of the **human** malaria mosquito Anopheles stephensi.
AUTHOR: Suwan N; Wilkinson M C; Crampton J M; Bates P A
CORPORATE SOURCE: Division of Molecular Biology and Immunology, Liverpool School of Tropical Medicine, School of Biological Sciences, University of Liverpool, Liverpool, UK.
SOURCE: Insect molecular biology, (2002 Jun) 11 (3) 223-32. Journal code: 9303579. ISSN: 0962-1075.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ269470; GENBANK-AJ269471
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020510
Last Updated on STN: 20020725
Entered Medline: 20020724

AB Full-length cDNA **clones** encoding D7 (AnsD7) and D7-related (AnsD7r1) **secreted salivary** gland proteins were isolated from Anopheles stephensi. Corresponding proteins were separated by SDS-PAGE and analysed by N-terminal sequencing, which also identified a second D7-related protein (AnsD7r2). AnsD7 encodes a protein of 37 kDa, AnsD7r1 of 18 kDa, and AnsD7r2 of 16 kDa. Polyclonal antibodies against **recombinant** AnsD7 showed immunological cross-reactivity with the D7-related proteins, and alignment demonstrated sequence similarity between the C-terminal region of AnsD7 and the D7-related proteins. AnsD7, AnsD7r1 and AnsD7r2 were major female-specific salivary gland proteins, and Western blotting, immunohistochemistry and immunogold labelling demonstrated **expression** was predominantly in the secretory cavities of the distal-lateral and median lobes. **Expression** and localization of D7 and D7-related proteins was similar in Plasmodium berghei-infected and uninfected mosquitoes.

L8 ANSWER 3 OF 14 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN DUPLICATE 2

ACCESSION NUMBER: 2002-08361 BIOTECHDS
TITLE: Polynucleotides encoding salivary proteins useful as anti-microbial agents;
vector-mediated gene transfer and **expression** in host cell for **recombinant** protein production and gene therapy
AUTHOR: ADLER D A; SHEPPARD P O
PATENT ASSIGNEE: ZYMOGENETICS INC
PATENT INFO: US 6331413 18 Dec 2001
APPLICATION INFO: US 1999-527345 17 Mar 1999
PRIORITY INFO: US 2000-527345 17 Mar 2000

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-096707 [13]

AB DERWENT ABSTRACT:

NOVELTY - Polynucleotides (I) derived from the 4q12-4q13 region of **human** chromosome 4 and encoding zsig63 polypeptides, a **secreted salivary** protein with anti-microbial activity, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) an isolated polynucleotide which hybridizes to the 4q12-4q13 region of **human** chromosome 4 under hybridization wash conditions of 0.1 x SSC to 2 x SSC, 0.1% SDS at 55-65 degreesC, and encodes a polypeptide that comprises a sequence of amino acid residues selected from: (a) a defined amino acid sequence (A1) given in the specification from amino acid number 16 (Arg) to amino acid number 37 (Ser); (b) the amino acid sequence A1 from amino acid number 38 (Leu) to amino acid number 126 (Ala); (c) the amino acid sequence A1 from amino acid number 127 (Pro) to amino acid number 219 (Gln); (d) the amino acid sequence A1 from amino acid number 16 (Arg) to amino acid number 219 (Gln); and/or (e) the amino acid sequence A1 from amino acid number 1 (Met) to amino acid number 219 (Gln); (2) an **expression** vector (II) comprising the following operably linked elements: (a) a transcription promoter; (b) a DNA segment wherein said segment hybridizes to the 4q12-4q13 region of **human** chromosome 4 under hybridization wash conditions of 0.1 x SSC to 2 x SSC, 0.1% SDS at 55-65 degreesC encoding a polypeptide comprising the amino acid sequence A1 from amino acid number 16 (Arg) to amino acid number 219 (Gln); and a transcription terminator; (3) a cultured cell (III) into which has been introduced the **expression** vector (II) (the cell **expresses** a polypeptide encoded by the DNA segment); (4) a DNA construct (IV) encoding a fusion protein, the DNA construct comprising: (a) a first DNA segment which hybridizes to the 4q12-4q13 region of **human** chromosome 4 under hybridization wash conditions of 0.1 x SSC to 2 x SSC, 0.1% SDS at 55-65 degreesC encoding a polypeptide selected from the following: (i) the amino acid sequence A1 from residue number 1 (Met) to residue number 15 (Ala); (ii) the amino acid sequence A1 from residue number 16 (Arg) to residue number 219 (Gln); and (b) at least one other DNA segment encoding an additional polypeptide (the first and other DNA segments are connected in-frame, and encode the fusion protein); and (5) a method (V) of producing a zsig63 polypeptide comprising: (a) culturing the cell (IV); and (b) isolating the zsig63 polypeptide produced by the cell.

BIOTECHNOLOGY - Preferred Polynucleotides: (I) Comprises a polynucleotide which hybridizes to the 4q12-4q13 region of **human** chromosome 4 under hybridization wash conditions of 0.1 x SSC to 2 x SSC, 0.1% SDS at 55-65 degreesC, selected from: (a) a defined polynucleotide sequence (N1) given in the specification from nucleotide 173 to nucleotide 238; (b) the polynucleotide sequence N1 from nucleotide 239 to nucleotide 505; (c) the polynucleotide sequence N1 from nucleotide 506 to nucleotide 784; (d) the polynucleotide sequenceN1 from nucleotide 173 to nucleotide 784; (e) the polynucleotide sequence N1 from nucleotide 128 to nucleotide 784; and (f) the polynucleotide sequence complementary to (a) through (e). Preferably, (I) comprises nucleotide 1 to nucleotide 657 of a defined nucleotide sequence (N2) given in the specification. Preferred **Expression** Vector: (II) Further comprises a secretory signal sequence operably linked to the DNA segment. Preparation: The nucleic acids were derived from the 4q12-4q13 region of **human** chromosome 4 by standard methodologies.

ACTIVITY - Antimicrobial. No biological data given.

MECHANISM OF ACTION - Gene therapy; Protein therapy.

USE - The polypeptides may be used for the **recombinant** production of anti-microbial proteins.

EXAMPLE - Scanning of a translated DNA database resulted in identification of an **expressed** sequence tag (EST) sequence

found to be a novel member of the adhesion family and designated zsig63. Confirmation of the EST sequence was made by sequence analyses of the cDNA from which the EST originated. This cDNA **clone** was obtained and sequenced using primers: ZC6768, ZC694, ZC7231, ZC7764a. The insert was 1 kb and was full-length.(1 pages)

L8 ANSWER 4 OF 14 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:95993 HCAPLUS
DOCUMENT NUMBER: 132:147626
TITLE: Protein and cDNA sequences of **human secreted salivary** protein zsig32, and uses thereof in diagnostic and therapeutic applications
INVENTOR(S): Sheppard, Paul O.
PATENT ASSIGNEE(S): Zymogenetics, Inc., USA
SOURCE: U.S., 36 pp., Cont.-in-part of U.S. Ser. No. 40,786.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6022847	A	20000208	US 1998-81180	19980519
US 6025197	A	20000215	US 1998-40786	19980318
US 2002082393	A1	20020627	US 1999-442952	19991118
US 2003176645	A1	20030918	US 2002-82649	20020222

PRIORITY APPLN. INFO.:

US 1997-41263P	P	19970319
US 1998-40786	A2	19980318
US 1998-81180	A1	19980519
US 1999-442952	B1	19991118

AB The invention provides protein and cDNA sequences of a **human secreted salivary** protein, designated zsig32. The protein of the invention has homol. to murine ventral prostate spermine-binding protein, Rattus norvegicus common salivary protein, and murine common salivary protein 1. The zsig32 protein comprises an adhesion motif and therefore may modulate adhesion and/or salivary gland function. The invention further relates to the use of zsig32 protein/gene in diagnostic and therapeutic applications, especially those directed toward adhesion, digestion, and/or mucosal maintenance.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 14 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000149824 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10684652
TITLE: Cellular phosphorylation of an acidic proline-rich protein, PRP1, a **secreted salivary** phosphoprotein.
AUTHOR: Drzymala L; Castle A; Cheung J C; Bennick A
CORPORATE SOURCE: Department of Biochemistry, University of Toronto, Toronto M5S 1A8, Canada.
SOURCE: Biochemistry, (2000 Feb 29) 39 (8) 2023-31.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000330
Last Updated on STN: 20020420
Entered Medline: 20000323
AB Phosphorylation of many **secreted salivary** proteins is

necessary for their biological functions. Identification of the kinase, which is responsible for in vivo phosphorylation, is complicated, because several of the protein phosphorylation sites conform both to the recognition sequence of casein kinase 2 (CK2) and Golgi kinase (G-CK), which both are found in the secretory pathway. This study was undertaken to determine the kinase recognition sequence in a secreted proline-rich salivary protein, PRP1, and thereby identify the responsible kinase. This was done by transfecting a **human** submandibular cell line, HSG, and a kidney cell line, HEK293, with **expression** vectors encoding wild-type or mutated PRP1. It was shown that phosphorylation occurred only at the same sites, Ser8 and 22, as in PRP1 purified from saliva. Phosphorylation at either site did not depend on the other site being phosphorylated. The sequence surrounding Ser8 has characteristics of both CK2 and G-CK recognition sequences, but destruction of the CK2 recognition site had no effect on phosphorylation, whereas no phosphorylation occurred if the G-CK recognition sequence was altered. The sequence surrounding Ser22 did not conform to any known kinase recognition sites. If Ser22 was mutated to Thr, no phosphorylation was seen, and a cluster of negatively charged residues at positions 27-29 was identified as part of the enzyme recognition site. Ser22 may be phosphorylated by a G-CK that recognizes an atypical substrate sequence or by a novel kinase. No difference in phosphorylation was seen between undifferentiated and differentiated HSG cells.

L8 ANSWER 6 OF 14 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:204678 HCAPLUS
DOCUMENT NUMBER: 133:190118
TITLE: Secretory proteins characteristic of environmental changes in cellular signal transduction: **expression** in oral fluid
AUTHOR(S): Mednieks, M. I.; Burke, J. C.; Sivakumar, T. P.; Hand, A. R.; Grindeland, R. E.
CORPORATE SOURCE: Department of Oral Biology, University of Illinois, Chicago, IL, 60302, USA
SOURCE: AIP Conference Proceedings (2000), 504(Space Technology and Applications International Forum, 2000, Pt. 1), 218-223
CODEN: APCPCS; ISSN: 0094-243X
PUBLISHER: American Institute of Physics
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Past studies have shown that both hypo- and hyper-gravity have significant consequences on a variety of tissues and organ systems. It is not known if the effects of environmental stimuli such as altered gravity are beneficial or detrimental, and if the effects can be prevented or reversed. Animal expts. from the Space Lab and Cosmos missions indicate that events that are mediated by cAMP, such as cellular responses to catecholamine and peptide hormone action, are significantly altered in a number of tissues as a consequence of space flight. A secretory cAMP-receptor protein (cARP), is present in saliva, and can serve as an indicator of individual responses to physiol. and environmental stress. Animal expts. have shown that the hypergravity component of space flight is a significant stress factor. In **humans**, cARP levels in each individual are constant under normal conditions, but elevated after acute stress. Addnl., the levels of cARP in **secreted saliva** can be compared to those in gingival crevicular fluid (GCF), which reflects the protein composition of serum. The ratio of cARP in saliva to that in GCF can be used as a measure of basal compared to hyper- or hypo-gravity values. An ultimate goal is to test hyper and zero G responses in **human** saliva to determine if cARP is a suitable index of acute and chronic stress. A miniaturized test kit for saliva collection has been designed. Samples can be collected and stored till analyses are carried out that will distinguish the effects of increased gravity from those of one and zero G. Such tests can serve as an individualized

monitoring system for physiol. responses either in space or on earth.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 7 OF 14 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:291214 HCAPLUS
DOCUMENT NUMBER: 131:72084
TITLE: In vivo analysis of secreted aspartyl proteinase **expression** in **human** oral candidiasis
AUTHOR(S): Naglik, Julian R.; Newport, George; White, Theodore C.; Fernandes-Naglik, Lynette L.; Greenspan, John S.; Greenspan, Deborah; Sweet, Simon P.; Challacombe, Stephen J.; Agabian, Nina
CORPORATE SOURCE: Department of Stomatology, University of California, San Francisco, CA, 94143-0422, USA
SOURCE: Infection and Immunity (1999), 67(5), 2482-2490
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Secreted aspartyl proteinases are putative virulence factors in Candida infections. Candida albicans possesses at least nine members of a SAP gene family, all of which have been sequenced. Although the **expression** of the SAP genes has been extensively characterized under laboratory growth conditions, no studies have analyzed in detail the in vivo **expression** of these proteinases in **human** oral colonization and infection. We have developed a reliable and sensitive procedure to detect C. albicans mRNA from whole saliva of patients with oral C. albicans infection and those with asymptomatic Candida carriage. The reverse transcription-PCR protocol was used to determine which of the SAP1 to SAP7 genes are **expressed** by C. albicans during colonization and infection of the oral cavity. SAP2 and the SAP4 to SAP6 subfamily were the predominant proteinase genes **expressed** in the oral cavities of both Candida carriers and patients with oral candidiasis; SAP4, SAP5, or SAP6 mRNA was detected in all subjects. SAP1 and SAP3 transcripts were observed only in patients with oral candidiasis. SAP7 mRNA **expression**, which has never been demonstrated under laboratory conditions, was detected in several of the patient samples. All seven SAP genes were simultaneously **expressed** in some patients with oral candidiasis. This is the first detailed study showing that the SAP gene family is **expressed** by C. albicans during colonization and infection in **humans** and that C. albicans infection is associated with the differential **expression** of individual SAP genes which may be involved in the pathogenesis of oral candidiasis.
REFERENCE COUNT: 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 8 OF 14 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 4
ACCESSION NUMBER: 1999190949 EMBASE
TITLE: Hypo- and hypersalivation induced by psychoactive drugs: Incidence, mechanisms and therapeutic implications.
AUTHOR: Szabadi E.; Tavernor S.
CORPORATE SOURCE: Prof. E. Szabadi, Division of Psychiatry, University of Nottingham, Queen's Medical Centre, Nottingham NC7 2UH, United Kingdom. elemer.szabadi@nottingham.ac.uk
SOURCE: CNS Drugs, (1999) 11/6 (449-466).
Refs: 221
ISSN: 1172-7047 CODEN: CNDREF
COUNTRY: New Zealand
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 037 Drug Literature Index
038 Adverse Reactions Titles
048 Gastroenterology

LANGUAGE: English
SUMMARY LANGUAGE: English
AB Psychoactive drugs can have profound effects on salivation: apart from affecting the amount of **saliva secreted** (i.e. causing either hypo- or hypersalivation), they may also modify the composition of the saliva. Hyposalivation leads to the subjective experience of 'dry mouth' (xerostomia) which, in addition to causing subjective discomfort, may be associated with dental caries and oral infections. On the other hand, hypersalivation leads to 'drooling' (sialorrhoea), which can be a source of social embarrassment and can be associated parotitis, choking and even aspiration pneumonia. Both xerostomia and sialorrhoea can impair patient compliance. Antidepressants [tricyclic antidepressants, noradrenaline (norepinephrine) reuptake inhibitors], lithium, antipsychotics of the phenothiazine class and α2- adrenoceptor agonists (e.g. **clonidine** and lofexidine) are commonly associated with hyposalivation and xerostomia, whereas both classical and novel antipsychotics can cause sialorrhoea. However, only clozapine-induced sialorrhoea, occurring in about 30% of patients treated with the drug, is of any major clinical significance. The salivary glands receive a dual sympathetic (noradrenergic) and parasympathetic (cholinergic) innervation, and psychoactive drugs may affect either the gland cells themselves or their neural control. The effects of psychoactive drugs on the autonomic control of salivation is mediated via their multiple actions at neuroreceptors and synaptic mechanisms. Blockade of muscarinic cholinoceptors and α1- adrenoceptors, inhibition of noradrenaline uptake and stimulation of α2- adrenoceptors may lead to hyposalivation, whereas stimulation of muscarinic cholinoceptors and dopamine D2 receptors, blockade of α2-adrenoceptors and depletion of noradrenaline from central stores may result in hypersalivation. It is important that the clinician is familiar with the effect of each class of drug on salivation, so that he/she can mitigate these by the judicious choice of drug and dosage schedule, and, if necessary, by providing symptomatic treatment for these distressing, and occasionally dangerous, adverse effects.

L8 ANSWER 9 OF 14 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 5
ACCESSION NUMBER: 97334015 EMBASE
DOCUMENT NUMBER: 1997334015
TITLE: Major salivary gland dysfunction in patients with hematological malignancies receiving interleukin-2-based immunotherapy post-autologous blood stem cell transplantation (ABSCT).
AUTHOR: Nagler A.; Nagler R.; Ackerstein A.; Levi S.; Marmary Y.
CORPORATE SOURCE: Dr. A. Nagler, Dept. of Bone Marrow Transplantation, Hadassah University Hospital, POB 12000, Jerusalem 91120, Israel
SOURCE: Bone Marrow Transplantation, (1997) 20/7 (575-580).
Refs: 32
ISSN: 0268-3369 CODEN: BMTRE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 011 Otorhinolaryngology
016 Cancer
025 Hematology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
038 Adverse Reactions Titles
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Interleukin-2 (IL-2) is known to cause xerostomia and skin manifestations similar to graft-versus-host disease (GVHD). We therefore evaluated major salivary gland function in patients with hematological malignancies treated with IL-2 and interferon-α (IFN-α) after ABSCT. Eleven

patients (seven male, four female) of median age 40 (24-47) were evaluated, seven with non-Hodgkin lymphoma (NHL); one with Hodgkin's disease (HD) and three with acute myelogenous leukemia (AML). Parotid and submandibular salivary gland function was assessed before, during and after IL2/IFN-α administration by evaluation of the salivary flow rate and the composition of **secreted saliva**.
Significant reductions in both the resting and stimulated parotid and submandibular salivary flow rates were observed during IL-2/IFN-α immunotherapy compared with the pre- and post-therapy values ($P < 0.01$), while no hyposalivation was observed in the control patients who underwent ABSCT and did not received IL-2. Sialochemical evaluation revealed a significant increase in potassium concentration (24.4 ± 0.6 mEq/l to 28.9 ± 1.4 mEq/l) and a significant decrease in sodium concentration (6.7 ± 2.1 mEq/l to 3.3 ± 1.0 mEq.l) ($P < 0.05$) in the stimulated parotid gland **saliva secreted** during IL-2/IFN-α administration. Salivary protein concentrations were not altered by the IL-2/IFN-α immunotherapy. Similar changes were previously observed in mice and **humans** with chronic GVHD. We conclude that IL-2 immunotherapy induces major salivary gland dysfunction in **humans**, similar to our previous observations in patients with chronic GVHD, which may indicate similar pathophysiologic mechanisms.

L8 ANSWER 10 OF 14 MEDLINE on STN
ACCESSION NUMBER: 94249000 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8191514
TITLE: [The bilateral autonomy of enzyme secretion by **human** salivary glands].
O bilateral'noi avtonomnosti sekretsii fermentov sliunnymi zhelezami cheloveka.
AUTHOR: Korot'ko G F; Kadirov Sh
SOURCE: Stomatologiia, (1994 Jan-Mar) 73 (1) 26-8.
Journal code: 0412072. ISSN: 0039-1735.
PUB. COUNTRY: RUSSIA: Russian Federation
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Dental Journals; Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940629
Last Updated on STN: 19990129
Entered Medline: 19940617

AB **Saliva secreted** by right and left parotid glands was collected using Leshley-Krasnogosky's capsules. Salivary secretion and aminolytic activity were found to be higher on the chewing side than on the contralateral. Bilateral differences in salivary gland secretion of alkaline and acid phosphatases and pepsinogen were less **expressed**. Salivary secretion in right-handed and left-handed subjects and in patients with hemiplegias after brain stroke was studied by citric acid stimulation. Bilateral asymmetry of enzymic secretion was revealed, particularly marked in the patients: not only the volume of salivary production and enzyme debit, but salivary enzymic activity on the side of motor paralysis were lower than on intact side. Bilateral functional asymmetry of secretion was more manifest for amylase than for other enzymes whose origin is largely influenced by recretion process.

L8 ANSWER 11 OF 14 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 93085022 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1452889
TITLE: Effect of age on immunoglobulin content and volume of **human** labial gland saliva.
AUTHOR: Smith D J; Joshipura K; Kent R; Taubman M A
CORPORATE SOURCE: Department of Immunology, Forsyth Dental Center, Boston, Massachusetts 02115.
CONTRACT NUMBER: DE-06153 (NIDCR)
DE-07009 (NIDCR)

SOURCE: Journal of dental research, (1992 Dec) 71 (12) 1891-4.
Journal code: 0354343. ISSN: 0022-0345.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals; Priority Journals
ENTRY MONTH: 199301
ENTRY DATE: Entered STN: 19930129
Last Updated on STN: 20000303
Entered Medline: 19930107

AB Stimulated lower labial (LLGF) and parotid salivary volumes and IgG, IgA, and IgM concentrations were measured in 264 subjects whose ages ranged from 17 to 76 years. A significant ($p < 0.001$) age-related decline in LLGF output was observed for subjects over this age range. Sixty-three percent of the subjects in the 18-20-year-old group (n = 46) secreted at least 10 microL of labial saliva in a 7-10-minute period, while approximately 70% of the subjects in the two oldest groups (61-70 and 71-76 years old) secreted less than 1 microL of LLGF during this time period (n = 64). No significant gender-based differences occurred in the volumes of labial **saliva secreted**. Stimulated parotid salivary flow showed no age-related trend in these subjects. Lower labial gland salivary IgA concentrations in an older population (mean age +/- SD = 55.6 yr +/- 1.3) were significantly lower ($p < 0.025$) than IgA concentrations in a younger population (20.7 yr +/- 0.8), when IgA was **expressed** as microgram/mL LLGF collected. Immunoglobulin A concentrations in parotid saliva and IgG and IgM concentrations in labial and parotid saliva were not significantly different when the two age populations were compared. These data suggest that the physiological and immunological potential of labial gland saliva may decrease with age.

L8 ANSWER 12 OF 14 MEDLINE on STN
ACCESSION NUMBER: 91321832 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1863431
TITLE: Enamel softening with Coca-Cola and rehardening with milk or saliva.
AUTHOR: Gedalia I; Dakuar A; Shapira L; Lewinstein I; Goultschin J; Rahamim E
CORPORATE SOURCE: Hadassah School of Dental Medicine, Hebrew University, Jerusalem.
SOURCE: American journal of dentistry, (1991 Jun) 4 (3) 120-2.
Journal code: 8806701. ISSN: 0894-8275.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
ENTRY MONTH: 199109
ENTRY DATE: Entered STN: 19910929
Last Updated on STN: 19970203
Entered Medline: 19910912

AB Rehardening effects by cow's milk and by **secreted saliva** were investigated, in situ, following softening of **human** enamel with an acidic beverage (Coca-Cola). Volunteers wearing orthodontic removable appliances participated in the study. The intra-oral test was chosen for measuring microhardness of enamel slabs inserted into the dental appliance. The softening and the rehardening degrees were defined as the alterations between initial- and experimental-microhardness value at the enamel surface. In addition, SEM photos were prepared from the initial and experimental stages. Exposure of enamel slabs to the acidic beverage during 1 hour had a softening effect as **expressed** by the hardness decrease and visualized by the SEM photo. Rehardening effects following milk or saliva exposures respectively were evident, presumably due to deposited organic and mineral material on the enamel surface.

L8 ANSWER 13 OF 14 MEDLINE on STN
ACCESSION NUMBER: 87309160 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3305627
TITLE: Structural and genetic aspects of proline-rich proteins.
AUTHOR: Bennick A
SOURCE: Journal of dental research, (1987 Feb) 66 (2) 457-61. Ref: 49
Journal code: 0354343. ISSN: 0022-0345.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Dental Journals; Priority Journals
ENTRY MONTH: 198710
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19871007

AB Considerable advances have been made in the genetics of salivary proline-rich proteins (PRP). The genes for acidic, basic, and glycosylated PRP have been **cloned**. They code for precursor proteins that all have an acidic N-terminal followed by proline-rich repeat sequences. Structural studies on secreted proteins have demonstrated that not only acidic but also some basic PRPs have this general structure. It is possible that mRNA for different PRP may have originated from a single gene by differential mRNA splicing, but post-translational cleavages of the primary translation product apparently also occur. In vitro translation of salivary gland mRNA results in a single precursor protein for acidic PRP. Such in vitro translated protein can be cleaved by salivary kallikrein, giving rise to two commonly secreted acidic PRPs, and kallikrein or kallikrein-like enzymes may be responsible for other post-translational cleavages of PRPs. Acidic as well as some basic PRPs are phosphorylated. A protein kinase has been demonstrated in salivary glands which phosphorylates the PRPs and other **secreted salivary** proteins in a cAMP and Ca2+-calmodulin-independent manner. Knowledge of the conformation of PRPs is limited. There is no conclusive evidence of polyproline-like structure in the proline-rich part of PRPs. Ca2+ binding studies on acidic PRPs indicate that there is interaction between the Ca2+ binding N-terminal end and the proline-rich C-terminal part. This interaction is relieved by modification of arginine side-chains. 1H, 32P, and 43Ca NMR studies have further elucidated the conformation of acidic PRPs in solution.(ABSTRACT TRUNCATED AT 250 WORDS)

L8 ANSWER 14 OF 14 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 7
ACCESSION NUMBER: 83189251 EMBASE
DOCUMENT NUMBER: 1983189251
TITLE: The chronically reserpinized rat as a model for cystic fibrosis: Alterations in the mucus-secreting sublingual gland.
AUTHOR: Ricardo Martinez J.; Bylund D.B.; Mawhinney T.; et al.
CORPORATE SOURCE: Dep. Child Health, Univ. Missouri Sch. Med., Columbia, MO, United States
SOURCE: Pediatric Research, (1983) 17/7 (523-528).
CODEN: PEREBL
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 007 Pediatrics and Pediatric Surgery
015 Chest Diseases, Thoracic Surgery and Tuberculosis
030 Pharmacology
004 Microbiology
005 General Pathology and Pathological Anatomy
023 Nuclear Medicine
037 Drug Literature Index

LANGUAGE: English

AB The chronic administration of reserpine (0.5 mg/kg body weight, daily for 7 days) increases the density of adrenergic receptors in the sublingual gland of the rat. The B(max) values (in pmole/g tissue) were 5.8 ± 0.9 for [3H]-prazosin, 22 ± 4 for [3H]-**clonidine** and 11.9 ± 1.7 for [3H]-dihydroalprenolol. These radioligands are used, respectively, for the study of α1, α2 and β-adrenergic receptors and the B(max) values indicated are 181, 226, and 331%, respectively, of the corresponding values in control sublingual glands. The increase in the density of α1-adrenergic receptors was accompanied by the development of a clear secretory response to norepinephrine and phenylephrine, as judged by the ability to release K+ in vitro. This response, which was not observed in control sublingual glands, amounted to 21.9 ± 2.5 and 16.8 ± 3.1% of the tissue content of K+, respectively, for the two agonists. Neither the density of muscarinic cholinergic receptors (B(max) = 106% of control value) nor the extent of K+ release elicited by carbamylcholine was modified by the drug treatment, when compared to those observed in control glands. In vivo, the volume of sublingual **saliva secreted** in response to i.v. infusions of acetylcholine was significantly reduced in the treated animals to 45% of that secreted by control rats. This was accompanied by increases in salivary concentrations of protein-bound carbohydrates, with no change in the individual carbohydrate ratios. No significant changes in salivary electrolyte concentrations (Na+, K+, Ca++) were observed in sublingual saliva of reserpine-treated rats. In summary, reserpine administration alters adrenergic receptor density and sensitivity in the rat sublingual gland and modified its secretory responses both in vitro and in vivo. The chronic administration of reserpine to rats causes widespread exocrine gland disturbances resembling those of cystic fibrosis. The results of this study show that the drug treatment also affects a mucus-secreting salivary gland and support the view that the reserpine-treated rat is a useful experimental model of the **human** disease.

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     (FILE 'HOME' ENTERED AT 15:56:47 ON 20 JUL 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 15:57:13 ON 20 JUL 2004
L1             8 S "ZSIG63"
L2             5 DUP REM L1 (3 DUPLICATES REMOVED)
L3        230476 S SALIVA?
L4           622 S SECRETED (A)L3
L5           171 S HUMAN AND L4
L6       6615667 S CLON? OR EXPRESS? OR RECOMBINANT
L7            28 S L5 AND L6
L8            14 DUP REM L7 (14 DUPLICATES REMOVED)

=> s "anti-microbial" or "anti-inflammatory" or "immunomodulatory"
L9       256214 "ANTI-MICROBIAL" OR "ANTI-INFLAMMATORY" OR "IMMUNOMODULATORY"

=> s l5 and l9
L10            4 L5 AND L9

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11            2 DUP REM L10 (2 DUPLICATES REMOVED)

=> d 1-2 ibib ab

L11   ANSWER 1 OF 2  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-01837  BIOTECHDS
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TITLE: Novel **secreted salivary** polypeptide, zsig63, useful as antimicrobial agent for treating microbial infection, dental carries, periodontal disease, thrush gastrointestinal disease, and for aiding digestion; recombinant protein production and agonist and antagonist use in disease therapy and gene therapy

AUTHOR: ADLER D A; SHEPPARD P O
PATENT ASSIGNEE: ADLER D A; SHEPPARD P O

PATENT INFO: US 2002090677 11 Jul 2002
APPLICATION INFO: US 2001-923236 3 Aug 2001
PRIORITY INFO: US 2001-923236 3 Aug 2001; US 1999-124820 17 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-642378 [69]

AB DERWENT ABSTRACT:

NOVELTY - An isolated **secreted salivary** polypeptide (I) designated as zsig63, comprising an amino acid sequence 90% identical to a sequence (S1) of 1 (Met)-219 (Gln) fully defined in the specification, 16 (Arg)-37 (Ser), 38 (Leu)-126 (Ala), 127 (Pro)-219 (Gln) or 16 (Arg)-219 (Gln) of (S1), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I); (2) an expression vector (III) comprising a transcription promoter, a DNA segment encoding a zsig63 polypeptide comprising an amino acid sequence that is 90% identical to (S1) from amino acid 16 (Arg)-219 (Gln) and a transcription terminator, operably linked to each other; (3) a cultured cell (IV) containing (III), expresses a polypeptide encoded by the DNA segment; (4) a DNA construct (V) encoding a fusion protein, comprises a first DNA segment encoding a polypeptide comprising an amino acid sequence of 1 (Met)-15 (Ala), 16 (Arg)-37 (Ser), 38 (Leu)-126 (Ala), 127 (Pro)-219 (Gln) or 16 (Arg)-219 (Gln) of (S1), and at least one other DNA segment encoding an additional polypeptide, where the first and other DNA segments are connected in-frame and encode the fusion protein; (5) a fusion protein produced by culturing a host cell comprising a vector having transcriptional promoter, (V), a transcriptional terminator, operably linked to each other and recovering the protein encoded by the DNA segment, is introduced; (6) producing (I); (7) detecting in a test sample, the presence of an agonist or antagonist of zsig63 protein activity, involves transfecting a zsig63-responsive cell, with a reporter gene construct that is responsive to a zsig63-stimulated cellular pathway and producing a zsig63 polypeptide by the culturing the cell, and adding the zig63 polypeptide to the cell in the presence and absence of a test sample and comparing levels of response to the zsig63 polypeptide, in the presence and absence of the test sample, by a biological or biochemical assay and determining from the comparison, the presence of the agonist or antagonist of zsig63 activity in the test sample; (8) producing an antibody to zsig63 polypeptide, involves inoculating animal with polypeptides consisting of 9 to 204 amino acids, the polypeptide consist of a contiguous sequence of amino acids in (S1) from amino acid 16 (Ala) to amino acid number 219 (Gln), (I), or a polynucleotide comprising 14 (Phe)-19 (Arg), 16 (Arg)-21 (Phe), 24 (Gly)-29(Asp), 25 (Glu)-30(Asp), 187 (Glu)-192 (Glu), 24 (Gly)-33 (Pro), 17 (Lys)-33 (Pro), 66 (Thr)-73 (Pro), 102 (Pro)-108 (Gly), 180 (Ala)-197 (Glu), 202 (Lys)-215 (Gly), 190 (Ala)-215 (Glu) of (S1), where the polypeptide elicits an immune response in the animal to produce the antibody, and isolating the antibody from the animal; and (9) an antibody (Ab) produced by the above method which binds to a zsig63 polypeptide or (I).

WIDER DISCLOSURE - Also disclosed are: (1) a zig63 polypeptide-encoding polynucleotides comprising a sequence of 657 nucleotides fully defined in the specification; (2) fragments of a sequence of 1008 nucleotides fully defined in the specification; (3) orthologs, variant or functional fragment of (I) and (II); (4) nucleic acid molecule encoding functional fragment of (I); (5) a pharmaceutical composition comprising (I); and (6) reagent comprising zsig63 gene, a

probe comprising zsig63 DNA or RNA or a subsequence for diagnostic application.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) and isolating (I) produced by the cell. Preferred Vector: (III) further comprises a secretory signal sequence operably linked to the DNA segment. Preferred Antibody: Ab is a monoclonal antibody.

ACTIVITY - Antibacterial; Fungicide; Virucide; Antiinflammatory; Antiarteriosclerotic; Vasotropic; Anorectic. No biological data provided.

MECHANISM OF ACTION - Gene therapy; Modulator of (I). No supporting data provided.

USE - (I) is useful as a host defense polypeptide, immune modulating factor, antipathogenic polypeptide, cell-cell signaling molecule, growth factor, cytokine, or as secreted extracellular matrix associated proteins with growth factor hormone activity. (I) is useful for treating conditions associated with pathological microbes, including bacterial, fungal, and viral infections, for treating dental carries (tooth decay), periodontal disease, thrush, and gastrointestinal disease, for treating urinary tract infection, vaginal infection, and for preventing infection in skin and other epithelial wounds. (I) is useful for establishing normal microflora and protect against pathogenic colonization and invasion, for treating chronic tissue damage e.g. damage in extremities associated with diabetes, and useful as **anti-inflammatory** agents. (I) is useful as a marker of lung dysfunction, salivary gland dysfunction, or dysfunction of prostate gland. (I) is therapeutically useful for aiding digestion. (I) is useful as a research reagent for the expansion of cultured cells, to prepare antibodies that bind to zsig63 epitopes, peptides or polypeptides, as antigen to inoculate an animal for eliciting an immune response. zsig63 is useful for identifying cells, tissues or cell lines which respond to zsig63-stimulated pathway, or for identifying inhibitors of its activity. (I) is useful for studies to isolate mesenchymal stem cells and myocyte or other progenitor cells both in vivo and ex vivo, and serve as an additional cell surface or secreted marker associated with stage-specific expression of a tissue. (II) is useful in gene therapy for increasing or inhibiting zsig63 activity, for detecting abnormalities on **human** chromosome 4 associated with disease or other **human** traits and as diagnostics in forensic DNA profiling. (I) or Ab is useful for studying chemoattraction of monocytes, for studying the activity of the melanocortin family of receptors, for studying or evaluating ligand or putative ligand binding and/or ion flux (calcium flux, potassium flux, sodium flux) regulation or modulation, for studying cytocidal activity against mammalian cells, as cell culture reagent in in vitro studies of exogenous microorganism infection, and in in vivo animal models of infection. (I) or Ab is useful for identifying and isolating receptors for zsig63. Ab is useful for detecting zsig63 polypeptides in the serum or tissue biopsy of a patient undergoing evaluation for salivary gland function or dysfunction. Ab is useful for tagging cells that express zsig63, for isolating zsig63 for diagnostic assays for determining circulating levels of zsig63 polypeptides, for detecting or quantitating soluble zsig63 as marker of underlying pathology or disease, in analytical methods employing fluorescence activated cell sorting (FACS), for screening expression libraries, for generating anti-idiotypic antibodies, as neutralizing antibodies or as antagonists to block zsig63 activity in vitro or in vivo, and in in vitro detection of denatured zsig63 or its fragments in assays. (I), (II) or Ab is useful for stimulating proliferation or differentiation of cardiac myocytes, for proliferation or differentiation of adipocytes and for inhibiting chondrosarcomas, atherosclerosis, restenosis and obesity.

ADMINISTRATION - Pharmaceutical composition comprising (I) is administered through topical, inhalation, parenteral, intravenous or subcutaneous route. Dosage not specified.

EXAMPLE - Full-length zsig63 was obtained using an expressed sequence tag (EST) sequence. Scanning of a translated DNA database resulted in identification of EST sequence found to be a novel member of

the adhesion family and designated zsig63. Confirmation of EST sequence was made by sequence analyses of the cDNA from the EST originated. This cDNA clone was obtained and sequenced using the ZC6768 (gcaattaaccctcactaaagggaac), ZC694 (taatacgactcactataggg), ZC7231 (ttttttttttttttttttttttttv), ZC7764a (ttttttttttttttttttttttta). The insert was about 1 kb and was full-length. (33 pages)

L11 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
ACCESSION NUMBER: 2002:143438 BIOSIS
DOCUMENT NUMBER: PREV200200143438
TITLE: **Secreted salivary** ZSIG63 Polypeptide.
AUTHOR(S): Adler, David A. [Inventor, Reprint author]; Sheppard, Paul O. [Inventor]
CORPORATE SOURCE: Bainbridge Island, WA, USA
ASSIGNEE: ZymoGenetics, Inc.
PATENT INFORMATION: US 6331413 December 18, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 18, 2001) Vol. 1253, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Feb 2002
Last Updated on STN: 26 Feb 2002
AB The present invention relates to polynucleotide and polypeptide molecules for zsig63, a novel **secreted salivary** protein. The polypeptides, and polynucleotides encoding them, may exhibit **anti -microbial** activity and may be used in the study or treatment of microbial infections. The polynucleotides encoding zsig63, are located on chromosome 4, and can be used to identify a region of the genome associated with **human** disease states. The present invention also includes antibodies to the zsig63 polypeptides.

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E1            1     ADLER CYRIL/AU
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E3          240 --> ADLER D A/AU
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E5           51     ADLER D C/AU
E6          218     ADLER D D/AU
E7           16     ADLER D E/AU
E8           52     ADLER D G/AU
E9           11     ADLER D H/AU
E10           6     ADLER D I/AU
E11          41     ADLER D J/AU
E12           7     ADLER D K/AU

=> s e3
L12         240 "ADLER D A"/AU

=> e sheppard p o/au
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E6            1     SHEPPARD P T/AU
E7           35     SHEPPARD P W/AU
E8            6     SHEPPARD PATRICIA/AU
E9           12     SHEPPARD PATRICIA C/AU
E10           1     SHEPPARD PATRICIA K/AU
E11           1     SHEPPARD PATRICIA KRIM/AU
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E12          19     SHEPPARD PAUL/AU

=> s e3
L13         204 "SHEPPARD P O"/AU

=> s l12 or l13
L14         439 L12 OR L13

=> d his

     (FILE 'HOME' ENTERED AT 15:56:47 ON 20 JUL 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 15:57:13 ON 20 JUL 2004
L1             8 S "ZSIG63"
L2             5 DUP REM L1 (3 DUPLICATES REMOVED)
L3        230476 S SALIVA?
L4           622 S SECRETED (A)L3
L5           171 S HUMAN AND L4
L6       6615667 S CLON? OR EXPRESS? OR RECOMBINANT
L7            28 S L5 AND L6
L8            14 DUP REM L7 (14 DUPLICATES REMOVED)
L9        256214 S "ANTI-MICROBIAL" OR "ANTI-INFLAMMATORY" OR "IMMUNOMODULATORY"
L10            4 S L5 AND L9
L11            2 DUP REM L10 (2 DUPLICATES REMOVED)
                 E ADLER D A/AU
L12          240 S E3
                 E SHEPPARD P O/AU
L13          204 S E3
L14          439 S L12 OR L13

=> s l5 and l14
L15            2 L5 AND L14

=> d 1-2 ibib ab

L15   ANSWER 1 OF 2  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-01837  BIOTECHDS
TITLE:            Novel secreted salivary polypeptide,
                  zsig63, useful as antimicrobial agent for treating microbial
                  infection, dental carries, periodontal disease, thrush
                  gastrointestinal disease, and for aiding digestion;
                     recombinant protein production and agonist and antagonist
                     use in disease therapy and gene therapy
AUTHOR:           ADLER D A; SHEPPARD P O
PATENT ASSIGNEE:  ADLER D A; SHEPPARD P O
PATENT INFO:      US 2002090677 11 Jul 2002
APPLICATION INFO: US 2001-923236 3 Aug 2001
PRIORITY INFO:    US 2001-923236 3 Aug 2001; US 1999-124820 17 Mar 1999
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2002-642378 [69]
AB    DERWENT ABSTRACT:
      NOVELTY - An isolated secreted salivary polypeptide
      (I) designated as zsig63, comprising an amino acid sequence 90% identical
      to a sequence (S1) of 1 (Met)-219 (Gln) fully defined in the
      specification, 16 (Arg)-37 (Ser), 38 (Leu)-126 (Ala), 127 (Pro)-219 (Gln)
      or 16 (Arg)-219 (Gln) of (S1), is new.
           DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
      following: (1) an isolated polynucleotide (II) encoding (I); (2) an
      expression vector (III) comprising a transcription promoter, a DNA
      segment encoding a zsig63 polypeptide comprising an amino acid sequence
      that is 90% identical to (S1) from amino acid 16 (Arg)-219 (Gln) and a
      transcription terminator, operably linked to each other; (3) a cultured
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cell (IV) containing (III), expresses a polypeptide encoded by the DNA segment; (4) a DNA construct (V) encoding a fusion protein, comprises a first DNA segment encoding a polypeptide comprising an amino acid sequence of 1 (Met)-15 (Ala), 16 (Arg)-37 (Ser), 38 (Leu)-126 (Ala), 127 (Pro)-219 (Gln) or 16 (Arg)-219 (Gln) of (S1), and at least one other DNA segment encoding an additional polypeptide, where the first and other DNA segments are connected in-frame and encode the fusion protein; (5) a fusion protein produced by culturing a host cell comprising a vector having transcriptional promoter, (V), a transcriptional terminator, operably linked to each other and recovering the protein encoded by the DNA segment, is introduced; (6) producing (I); (7) detecting in a test sample, the presence of an agonist or antagonist of zsig63 protein activity, involves transfecting a zsig63-responsive cell, with a reporter gene construct that is responsive to a zsig63-stimulated cellular pathway and producing a zsig63 polypeptide by the culturing the cell, and adding the zig63 polypeptide to the cell in the presence and absence of a test sample and comparing levels of response to the zsig63 polypeptide, in the presence and absence of the test sample, by a biological or biochemical assay and determining from the comparison, the presence of the agonist or antagonist of zsig63 activity in the test sample; (8) producing an antibody to zsig63 polypeptide, involves inoculating animal with polypeptides consisting of 9 to 204 amino acids, the polypeptide consist of a contiguous sequence of amino acids in (S1) from amino acid 16 (Ala) to amino acid number 219 (Gln), (I), or a polynucleotide comprising 14 (Phe)-19 (Arg), 16 (Arg)-21 (Phe), 24 (Gly)-29(Asp), 25 (Glu)-30(Asp), 187 (Glu)-192 (Glu), 24 (Gly)-33 (Pro), 17 (Lys)-33 (Pro), 66 (Thr)-73 (Pro), 102 (Pro)-108 (Gly), 180 (Ala)-197 (Glu), 202 (Lys)-215 (Gly), 190 (Ala)-215 (Glu) of (S1), where the polypeptide elicits an immune response in the animal to produce the antibody, and isolating the antibody from the animal; and (9) an antibody (Ab) produced by the above method which binds to a zsig63 polypeptide or (I).

WIDER DISCLOSURE - Also disclosed are: (1) a zig63 polypeptide-encoding polynucleotides comprising a sequence of 657 nucleotides fully defined in the specification; (2) fragments of a sequence of 1008 nucleotides fully defined in the specification; (3) orthologs, variant or functional fragment of (I) and (II); (4) nucleic acid molecule encoding functional fragment of (I); (5) a pharmaceutical composition comprising (I); and (6) reagent comprising zsig63 gene, a probe comprising zsig63 DNA or RNA or a subsequence for diagnostic application.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) and isolating (I) produced by the cell. Preferred Vector: (III) further comprises a secretory signal sequence operably linked to the DNA segment. Preferred Antibody: Ab is a monoclonal antibody.

ACTIVITY - Antibacterial; Fungicide; Virucide; Antiinflammatory; Antiarteriosclerotic; Vasotropic; Anorectic. No biological data provided.

MECHANISM OF ACTION - Gene therapy; Modulator of (I). No supporting data provided.

USE - (I) is useful as a host defense polypeptide, immune modulating factor, antipathogenic polypeptide, cell-cell signaling molecule, growth factor, cytokine, or as secreted extracellular matrix associated proteins with growth factor hormone activity. (I) is useful for treating conditions associated with pathological microbes, including bacterial, fungal, and viral infections, for treating dental carries (tooth decay), periodontal disease, thrush, and gastrointestinal disease, for treating urinary tract infection, vaginal infection, and for preventing infection in skin and other epithelial wounds. (I) is useful for establishing normal microflora and protect against pathogenic colonization and invasion, for treating chronic tissue damage e.g. damage in extremities associated with diabetes, and useful as anti-inflammatory agents. (I) is useful as a marker of lung dysfunction, salivary gland dysfunction, or dysfunction of prostate gland. (I) is therapeutically useful for aiding digestion. (I) is useful as a research reagent for the expansion of cultured cells, to prepare antibodies that bind to zsig63 epitopes,

peptides or polypeptides, as antigen to inoculate an animal for eliciting an immune response. zsig63 is useful for identifying cells, tissues or cell lines which respond to zsig63-stimulated pathway, or for identifying inhibitors of its activity. (I) is useful for studies to isolate mesenchymal stem cells and myocyte or other progenitor cells both in vivo and ex vivo, and serve as an additional cell surface or secreted marker associated with stage-specific expression of a tissue. (II) is useful in gene therapy for increasing or inhibiting zsig63 activity, for detecting abnormalities on **human** chromosome 4 associated with disease or other **human** traits and as diagnostics in forensic DNA profiling. (I) or Ab is useful for studying chemoattraction of monocytes, for studying the activity of the melanocortin family of receptors, for studying or evaluating ligand or putative ligand binding and/or ion flux (calcium flux, potassium flux, sodium flux) regulation or modulation, for studying cytocidal activity against mammalian cells, as cell culture reagent in in vitro studies of exogenous microorganism infection, and in in vivo animal models of infection. (I) or Ab is useful for identifying and isolating receptors for zsig63. Ab is useful for detecting zsig63 polypeptides in the serum or tissue biopsy of a patient undergoing evaluation for salivary gland function or dysfunction. Ab is useful for tagging cells that express zsig63, for isolating zsig63 for diagnostic assays for determining circulating levels of zsig63 polypeptides, for detecting or quantitating soluble zsig63 as marker of underlying pathology or disease, in analytical methods employing fluorescence activated cell sorting (FACS), for screening expression libraries, for generating anti-idiotypic antibodies, as neutralizing antibodies or as antagonists to block zsig63 activity in vitro or in vivo, and in in vitro detection of denatured zsig63 or its fragments in assays. (I), (II) or Ab is useful for stimulating proliferation or differentiation of cardiac myocytes, for proliferation or differentiation of adipocytes and for inhibiting chondrosarcomas, atherosclerosis, restenosis and obesity.

ADMINISTRATION - Pharmaceutical composition comprising (I) is administered through topical, inhalation, parenteral, intravenous or subcutaneous route. Dosage not specified.

EXAMPLE - Full-length zsig63 was obtained using an expressed sequence tag (EST) sequence. Scanning of a translated DNA database resulted in identification of EST sequence found to be a novel member of the adhesion family and designated zsig63. Confirmation of EST sequence was made by sequence analyses of the cDNA from the EST originated. This cDNA clone was obtained and sequenced using the ZC6768 (gcaattaaccctcactaaagggaac), ZC694 (taatacgactcactataggg), ZC7231 (ttttttttttttttttttttttttv), ZC7764a (ttttttttttttttttttttttttta). The insert was about 1 kb and was full-length. (33 pages)

L15 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-08361 BIOTECHDS
TITLE: Polynucleotides encoding salivary proteins useful as anti-microbial agents;
vector-mediated gene transfer and expression in host cell for recombinant protein production and gene therapy
AUTHOR: **ADLER D A; SHEPPARD P O**
PATENT ASSIGNEE: ZYMOGENETICS INC
PATENT INFO: US 6331413 18 Dec 2001
APPLICATION INFO: US 1999-527345 17 Mar 1999
PRIORITY INFO: US 2000-527345 17 Mar 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-096707 [13]
AB DERWENT ABSTRACT:

NOVELTY - Polynucleotides (I) derived from the 4q12-4q13 region of **human** chromosome 4 and encoding zsig63 polypeptides, a **secreted salivary** protein with anti-microbial activity, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the

following: (1) an isolated polynucleotide which hybridizes to the 4q12-4q13 region of **human** chromosome 4 under hybridization wash conditions of 0.1 x SSC to 2 x SSC, 0.1% SDS at 55-65 degreesC, and encodes a polypeptide that comprises a sequence of amino acid residues selected from: (a) a defined amino acid sequence (A1) given in the specification from amino acid number 16 (Arg) to amino acid number 37 (Ser); (b) the amino acid sequence A1 from amino acid number 38 (Leu) to amino acid number 126 (Ala); (c) the amino acid sequence A1 from amino acid number 127 (Pro) to amino acid number 219 (Gln); (d) the amino acid sequence A1 from amino acid number 16 (Arg) to amino acid number 219 (Gln); and/or (e) the amino acid sequence A1 from amino acid number 1 (Met) to amino acid number 219 (Gln); (2) an expression vector (II) comprising the following operably linked elements: (a) a transcription promoter; (b) a DNA segment wherein said segment hybridizes to the 4q12-4q13 region of **human** chromosome 4 under hybridization wash conditions of 0.1 x SSC to 2 x SSC, 0.1% SDS at 55-65 degreesC encoding a polypeptide comprising the amino acid sequence A1 from amino acid number 16 (Arg) to amino acid number 219 (Gln); and a transcription terminator; (3) a cultured cell (III) into which has been introduced the expression vector (II) (the cell expresses a polypeptide encoded by the DNA segment); (4) a DNA construct (IV) encoding a fusion protein, the DNA construct comprising: (a) a first DNA segment which hybridizes to the 4q12-4q13 region of **human** chromosome 4 under hybridization wash conditions of 0.1 x SSC to 2 x SSC, 0.1% SDS at 55-65 degreesC encoding a polypeptide selected from the following: (i) the amino acid sequence A1 from residue number 1 (Met) to residue number 15 (Ala); (ii) the amino acid sequence A1 from residue number 16 (Arg) to residue number 219 (Gln); and (b) at least one other DNA segment encoding an additional polypeptide (the first and other DNA segments are connected in-frame, and encode the fusion protein); and (5) a method (V) of producing a zsig63 polypeptide comprising: (a) culturing the cell (IV); and (b) isolating the zsig63 polypeptide produced by the cell.

BIOTECHNOLOGY - Preferred Polynucleotides: (I) Comprises a polynucleotide which hybridizes to the 4q12-4q13 region of **human** chromosome 4 under hybridization wash conditions of 0.1 x SSC to 2 x SSC, 0.1% SDS at 55-65 degreesC, selected from: (a) a defined polynucleotide sequence (N1) given in the specification from nucleotide 173 to nucleotide 238; (b) the polynucleotide sequence N1 from nucleotide 239 to nucleotide 505; (c) the polynucleotide sequence N1 from nucleotide 506 to nucleotide 784; (d) the polynucleotide sequenceN1 from nucleotide 173 to nucleotide 784; (e) the polynucleotide sequence N1 from nucleotide 128 to nucleotide 784; and (f) the polynucleotide sequence complementary to (a) through (e). Preferably, (I) comprises nucleotide 1 to nucleotide 657 of a defined nucleotide sequence (N2) given in the specification. Preferred Expression Vector: (II) Further comprises a secretory signal sequence operably linked to the DNA segment. Preparation: The nucleic acids were derived from the 4q12-4q13 region of **human** chromosome 4 by standard methodologies.

ACTIVITY - Antimicrobial. No biological data given.

MECHANISM OF ACTION - Gene therapy; Protein therapy.

USE - The polypeptides may be used for the recombinant production of anti-microbial proteins.

EXAMPLE - Scanning of a translated DNA database resulted in identification of an expressed sequence tag (EST) sequence found to be a novel member of the adhesion family and designated zsig63. Confirmation of the EST sequence was made by sequence analyses of the cDNA from which the EST originated. This cDNA clone was obtained and sequenced using primers: ZC6768, ZC694, ZC7231, ZC7764a. The insert was 1 kb and was full-length.(1 pages)

=> d his

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 15:57:13 ON 20 JUL 2004
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L2             5 DUP REM L1 (3 DUPLICATES REMOVED)
L3        230476 S SALIVA?
L4           622 S SECRETED (A)L3
L5           171 S HUMAN AND L4
L6       6615667 S CLON? OR EXPRESS? OR RECOMBINANT
L7            28 S L5 AND L6
L8            14 DUP REM L7 (14 DUPLICATES REMOVED)
L9        256214 S "ANTI-MICROBIAL" OR "ANTI-INFLAMMATORY" OR "IMMUNOMODULATORY"
L10            4 S L5 AND L9
L11            2 DUP REM L10 (2 DUPLICATES REMOVED)
                 E ADLER D A/AU
L12          240 S E3
                 E SHEPPARD P O/AU
L13          204 S E3
L14          439 S L12 OR L13
L15            2 S L5 AND L14
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	Issue Date	Pages	Document ID	Title
1	20021121	32	US 20020173027 A1	Secreted salivary zsig63 polypeptide
2	20020711	33	US 20020090677 A1	Secreted salivary zsig63 polypeptide
3	20020627	33	US 20020081701 A1	Secreted salivary zsig63 polypeptide
4	20011218	29	US 6331413 B1	Secreted salivary ZSIG63 Polypeptide

	Issue Date	Pages	Document ID	Title
1	20031225	222	US 20030235820 A1	Novel methods of diagnosis of metastatic colorectal cancer, compositions and methods of screening for modulators of metastatic colorectal cancer
2	20031127	176	US 20030219875 A1	Albumin fusion proteins
3	20030123	40	US 20030017495 A1	Enterococcus faecalis polynucleotides and polypeptides
4	20020418	255	US 20020045737 A1	ENTEROCOCCUS FAECALIS POLYNUCLEOTIDES AND POLYPEPTIDES
5	20020910	146	US 6448043 B1	Enterococcus faecalis EF040 and uses therefor
6	20020430	73	US 6380362 B1	Polynucleotides, polypeptides expressed by the polynucleotides and methods for their use
7	20020101	227	US 6335170 B1	Gene expression in bladder tumors

	L #	Hits	Search Text
1	L1	4	"zsig63"
2	L2	7569	salivary
3	L3	124773	secret$3
4	L4	971	l2 same l3
5	L5	643561	clon$3 or express$3 or recombinant
6	L6	340	l4 same l5
7	L7	228866	adhesion
8	L8	7	l6 same l7

	Issue Date	Pages	Document ID	Title
1	20040715	26	US 20040137575 A1	Human 2-19 protein homologue, z219c
2	20040624	124	US 20040121380 A1	Novel polypeptides and nucleic acids encoding same
3	20040325	87	US 20040058862 A1	Novel polypeptides and nucleic acids encoding the same
4	20031120	88	US 20030216308 A1	Novel polypeptides and nucleic acids encoding same
5	20030918	62	US 20030176652 A1	Human and mouse beta-defensins, antimicrobial peptides
6	20030807	74	US 20030148485 A1	Novel polypeptides and nucleic acids encoding same
7	20030522	130	US 20030096952 A1	Novel proteins and nucleic acids encoding same
8	20030320	57	US 20030054539 A1	Endoglucanases
9	20030213	37	US 20030032792 A1	Human 2-19 protein homologue, z219a

	Issue Date	Pages	Document ID	Title
10	20020801	43	US 20020102657 A1	Ligands directed to the non-secretory component, non-stalk region of pIgR and methods of use thereof
11	20020110	28	US 20020004238 A1	Chimeric antigen-enterotoxin mucosal immunogens
12	20021022	75	US 6468547 B1	Enhancement of tumor cell chemosensitivity and radiosensitivity using single chain secretory antibodies
13	20020514	34	US 6388064 B1	Human 2-19 protein homologue, z219a
14	20020514	95	US 6387690 B1	Endoglucanases
15	20000606	25	US 6071735 A	Enzyme preparation with endoglucanase activity
16	19991214	93	US 6001639 A	Endoglucanases
17	19990706	27	US 5919691 A	Enzyme and enzyme preparation with endoglucanase activity
18	19981020	42	US 5824505 A	Vectors, transformed cells and process for the preparation of hirudin
19	19980106		US 5705355 A	Hirudin, pharmaceutical compositions comprising it and their use

	Issue Date	Pages	Document ID	Title
1	20021121	32	US 20020173027 A1	Secreted salivary zsig63 polypeptide
2	20020711	33	US 20020090677 A1	Secreted salivary zsig63 polypeptide
3	20020627	33	US 20020081701 A1	Secreted salivary zsig63 polypeptide
4	20011218	29	US 6331413 B1	Secreted salivary ZSIG63 Polypeptide

	L #	Hits	Search Text
1	L1	4	"zsig63"
2	L2	7569	salivary
3	L3	124773	secret$3
4	L4	971	l2 same l3
5	L5	643561	clon$3 or express$3 or recombinant
6	L6	340	l4 same l5
7	L7	228866	adhesion
8	L8	7	l6 same l7
9	L9	15544	ADLER SHEPPARD
10	L10	19	l6 and l9
11	L11	4	l1 and l9